

OBSERVATIONS AND EXPERIMENTS ON METHODS OF FERTILIZATION IN THE CHAETOGNATH *SAGITTA HISPIDA*

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For many years, the only laboratory-maintained chaetognath was *Spadella cephaloptera*, a member of the single benthic genus. Until the development of culture ability with *Sagitta hispida* (Reeve, 1970a), no extensive behavioral observations were possible in the pelagic genera, which are generally considered to constitute the second most important group of marine macroplankton. The process by which fertilization occurs in *Spadella*, involving a reciprocal transference of spermatophores between two individuals, has been extensively documented by Ghirardelli (*e.g.*, 1968). For the other genera, there exist scattered observations of a largely circumstantial nature which support the possibilities of both cross- and self-fertilization. These observations are reviewed in detail by Reeve and Cospér (1972) who noted that Hyman (1959) stated that "Self-fertilization is thus apparently the rule in *Sagitta*," (page 29) although Alvarino (1965) concluded for chaetognaths as a whole that "... it is generally accepted that cross-fertilization by copulation is the rule" (page 132). Since both reviewers indicated the dominance of the genus *Sagitta* within the phylum, these opinions are essentially contradictory. Ghirardelli (1968) preferred to conclude that one method does not necessarily exclude the other, even in the same species.

Foremost amongst the evidence for self-fertilization is the direct observation of the migration of spermatozoa from the seminal vesicle forward along the tail and into the seminal receptacle in isolated individuals of the species *Sagitta setosa* (Jägersten, 1940; Ghirardelli, 1968; Dallot, 1968). The last author was able to show that this resulted in the laying of fertile eggs in some cases. The evidence supporting cross-fertilization is based on observations of objects considered to be spermatophores attached to specimens from plankton samples (*e.g.*, Dallot, as reported to Ghirardelli, 1968; David, 1958) and the specialization of structure of seminal vesicles in some species, such as *S. bipunctata* where the seminal vesicle is surmounted by a small cup with "saw teeth" edges. Ghirardelli (1968) believed such structures might serve as copulatory organs whose form fits the female genital orifice of that species only, preventing mating between different species. Further circumstantial evidence for cross-fertilization in pelagic chaetognaths occurs in the observation of Murikami (1959) concerning specimens of *Sagitta crassa* which were found adjacent to each other with heads oriented in the opposite direction, presumably copulating. In addition, the fact that cross-fertilization is normal in the only species to be extensively studied alive (*Spadella cephaloptera*), and exclusive self-fertilization at least on an evolutionary time scale seems highly unlikely, suggests that extensive observation of pelagic species would be necessary before attempting to discount any possibility of cross-fertilization.

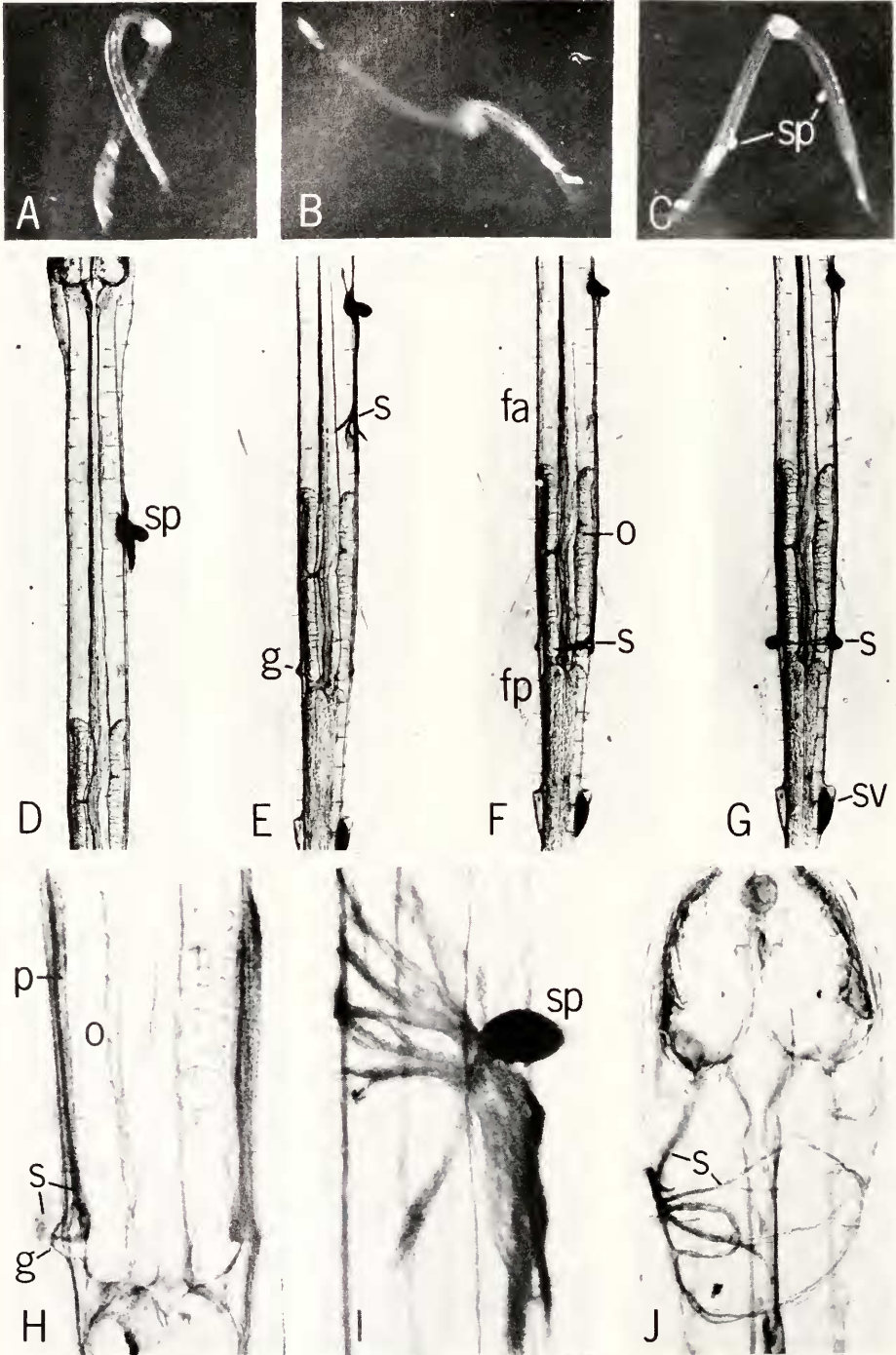


FIGURE 1.

The observations and experiments reported below show that both self- and cross-fertilization can occur in the same species and provide some indication of their relative frequency.

OBSERVATIONS AND RESULTS

Sagitta hispida Conant occurs in Biscayne Bay, adjacent to the laboratory, and can be maintained successfully in culture (Reeve, 1970a) by providing large aquaria, frequently changed, well aerated water and live zooplankton for food. Observations were made upon populations in rectangular acrylic or glass aquaria in volumes of 40 liters with a water depth exceeding 45 cm. Individuals and pairs were isolated in 250 ml containers.

Cross-fertilization

In laboratory culture acts of cannibalism by *Sagitta hispida* are frequently observed, especially in older animals. Usually the attacker seizes the head of its victim by means of its spines or "jaws" (rather than another part of the body) and proceeds to ingest it whole. When both animals are mature and hence of similar size, several minutes may elapse during which the pair engages in violent swimming motions. Often the animals separate, and swim off apparently unharmed, bearing a small dark attachment on the side of their bodies. Microscopical examination has shown this attachment to be a spermatophore packed with motile sperm.

We have each seen the initiation of attack once. In each case, two animals made several upward darting movements in close proximity to each other before becoming attached. In the first case, spermatophore transference occurred and the animals separated some 90 seconds later. In the second case, they detached within a few seconds without any transference. Rapid darting movements, in which two animals may swim closely together from the bottom to the top of the water column (45 cm) and back again within 5 seconds, are seen mostly in populations in which attachments are occurring. We suggest that it is a behavioral sequence associated with copulation. During the attachment phase of the encounter, the

FIGURE 1. (A-C) The copulatory "dance." Three separate pairs of animals attached at their head ends engaged in characteristic violent movements during which spermatophores were exchanged. In C, each of the pair has gained a spermatophore. (D-G) External migration of sperm. This sequence taken of a single animal shows (D) the newly implanted spermatophore from which sperm are beginning to move out, (E) a strong posteriorly directed stream moving along the lateral body wall immediately above the right anterior fin, (F) the arrival of the stream over the right gonopore where it begins to traverse the dorsal body wall towards the left gonopore until (G) accumulations of sperm build up over each gonopore. (H) The penetration of sperm through the left gonopore into the sperm pouch (which can be seen running anteriorly, exterior to the ovary containing eggs, by virtue of the sperm filling it.) Some sperm still remain outside of the body wall. (I) A spermatophore artificially attached on the dorsal body surface, which illustrates the migration of sperm streams to the lateral body wall on both sides rather than (as in *Spadella*) travelling along the dorsal body wall. (J) The remains of a spermatophore artificially attached immediately behind the head laterally on the collarete, streams from which have meandered in the region failing to establish a gonopore-oriented direction to their flow. Subsequent to this photograph, they became diffuse and disappeared. The approximate length of these mature animals is 9 mm and width of the trunk region 0.5 mm. Abbreviations are: fa—anterior fin; fp—posterior fin; g—gonopore; o—ovary; p—seminal pouch; s—sperm; sp—spermatophore; sv—seminal vesicle.

frequency and violence of the muscular movements of the partners is much more intense than in normal swimming movements. Attached head-on (Fig. 1 A-C), their bodies flex and twist in close proximity along their lengths as they move upwards in a spiralling motion followed by intervals of rest when they sink downwards.

At least 50 such encounters have been observed. Usually the encounter ended with both animals gaining and losing a spermatophore (Fig. 1 C). Only in situations where a single spermatophore was exchanged could its origin be in no doubt although we assume that all transferences were from the other animal. The moment of transference was never seen (the swimming movement appeared blurred when photographed with a flash of 1/250 sec duration). The site of attachment was always on the lateral trunk wall, often between the anterior and posterior fins, which is about one-third of the total body length anterior to the seminal vesicles. Although sometimes it varied in either direction, it was not to be found on the tail or head, or mid-dorsally or ventrally, nor on "the dorsal median line directly behind the ciliary loop" as in *Spadella* (Ghirardelli, 1968). No phenomenon has ever been seen in our populations in which animals were oriented head-to-tail, as occurs in *Spadella* while one is attached to a substrate, or as Murikami saw in *Sagitta crassa*. On one occasion three animals were observed attached at their head ends, and on separating at least two had lost and received spermatophores.

Immediately after receipt of a spermatophore the animal may be removed from the aquarium, placed in a small dish and viewed microscopically. Invariably, within the few seconds which elapse prior to examination, sperm are already streaming across the body surface (Fig. 1 D). Since no empty spermatophore is left behind, we believe that the "spermatophore" consists of the tightly packed sperms enveloped in a thin layer of material of presumably adhesive mucoid nature which disintegrates as the sperm stream through it. Ghirardelli (1968) refers to the phenomenon as the dissolving of the spermatophore. Although several streams radiate out in various directions at first (Fig. 1 E), only the stream which proceeds laterally in a posterior direction continues to move, and it does so until it reaches the female gonophore on that side (Fig. 1 F), which is situated close to the junction of the trunk and tail. Some of the sperm then travel around the circumference of the body to the gonopore on the opposite side, so that both pores have accumulations of sperm over them (Fig. 1 G). The sperm enter and migrate along the seminal receptacle or pouch which extends along the outer edge of the ovary (Fig. 1 H). The process by which internal fertilization of the eggs then occurs is described in detail by Ghirardelli (1968) who provided a series of photographs showing a pattern of sperm migration from the dorsal body wall behind the ciliary loop in *Spadella*. In this case, the sperm path bifurcates to reach both gonopores at the same time. In the series of photographs in Figure 1 D-G, the elapsed time from spermatophore attachment at the level of the anterior end of the anterior fin to entrance at the gonopores was 10 minutes. The rest of the sperm which had not reached the gonopores quickly lost motility and are presumably detached during swimming movements of the animal, because after a further 10 minutes there are none remaining on the outside of the body. This condition varies from *Spadella*, where nearly all the sperm travel to and enter the gonopores.

Self-fertilization

In the course of experimental work involving the measurement of mature live animals over a long period of time, five individuals were accidentally observed where sperm were emerging from a small rupture of the seminal vesicle anteriorly and migrating along the lateral edge of the tail, entering the gonopore on that side. Relative to the volume of sperm in the seminal vesicle, the sperm were very few in number. The animals subsequently died before laying eggs.

Self-insemination can be artificially induced by breaking open a full seminal vesicle using a very fine needle which causes the spermatophore to "pop" out. By confining the animal to a small dish in which the depth of water is only 2–4 mm, the spermatophore can usually be maneuvered to touch the body of the animal where it sometimes adheres. When placed on the lateral trunk wall, the sperm stream out according to the sequence of events described in the previous section. This procedure was performed only on animals which had been isolated before becoming mature, at a stage when seminal vesicles had not begun to develop, ovaries were tiny and contained only stage I eggs (both conditions which are known not to be reverted to after maturity in this species, Reeve, 1970b) and which were smaller than any mature individuals. This was to insure that there was no possibility that prior insemination could have taken place. A total of 41 animals which were inseminated by their own sperm in this manner laid eggs which developed into larvae. None laid infertile eggs. Most of the animals which did not survive to lay eggs died during or within hours of handling, many obviously damaged in the process.

In the preceding experiments, spermatophore adhesion was directed to the region where it occurs naturally in cross-fertilization. Some attempts were also made to place spermatophores on other parts of the body. Anterior to the ventral ganglion, including the region of the ciliary loop, sperm trails issued from the spermatophore in various directions but none travelled far back towards the gonopore (Fig. 1-J). Results were less clear when spermatophores were placed on the tail. No strong directional flows occurred, but the close proximity of the gonopore and the flicking of the tail fin during swimming attempts were both probably factors in permitting a few sperm to occasionally reach the gonopores. It was difficult to obtain adhesion of spermatophores dorsally or ventrally, and where this occurred, as in the dorso-lateral position of Figure 1-I, sperm trails would first orient themselves laterally often on both sides of the body before moving towards the gonopore. Unlike the condition in *Spadella*, therefore, sperm could not be induced to move down the mid-dorsal line of the body wall.

Relative frequency of self- and cross-fertilization

It was reported (Reeve, 1970b) that larvae could result from eggs laid by isolated individuals. Such occurrences are infrequent but since that study was concerned with estimates of fecundity based on egg-laying, quantitative data on fertility were not obtained. The maturation and laying of eggs in *Sagitta hispida* is not dependent on prior insemination and fertilization.

In a new series of experiments, 66 animals out of 86 isolated from immaturity as described above laid one or more batches of eggs amounting to a total of 186 batches. Only four batches (2%) resulted in the production of larvae which

themselves amounted to less than 17% of the number of eggs laid in those four batches. No batches were discarded until beyond the normal period required for hatching (36–48 hr) although fertile eggs would cleave within 10 minutes of being laid.

Since it is possible that the confinement of a normally planktonic 9-mm long animal in 250 ml could adversely influence its ability to produce offspring, a preliminary experiment was run in which 10 pairs of immature animals were utilized. Only three pairs survived long enough to produce a batch of eggs, all of which were fertile. This experiment was not expanded because of mortality problems probably aggravated by cannibalism. The following experiment, however, demonstrated the fact that larvae could be produced in close confinement.

This experiment was performed primarily to ascertain whether an animal once inseminated could store sperm for fertilization of subsequent batches of eggs. This ability is possessed by planktonic marine copepods (*e.g.*, Marshall and Orr, 1955). Observations suggested that sperm storage was unlikely because there were no visible signs of sperm in the seminal pouches on the day following insemination (using $\times 12$ magnification). Initially, their position can be seen even by the unaided eye.

Mature animals with well-developed ovaries were removed from aquarium populations and isolated. Of 32 animals isolated, 24 subsequently laid a batch of eggs, of which 10 were fertile and resulted in larvae only slightly fewer in number than eggs laid. A total of 23 further batches were laid by this group, all of which were infertile. In the case of those whose first batch was infertile, we presume that they had been removed from the population before insemination had occurred. There was no evidence that insemination conferred fertility on any but a single batch of eggs.

DISCUSSION

On the basis of our own observations of *Sagitta hispida*, we would agree with Alvaríño that cross-fertilization by copulation is the rule. Strictly, most observations in the literature have concerned insemination rather than fertilization. Our indication of successful fertilization was the hatching of a larva, but we have no information on the viability or ultimate fecundity of such larvae. In *Sagitta hispida* our observations suggest that larvae are normally produced following insemination during copulation which can sometimes be proved to be cross-insemination (where only a single spermatophore is involved). Self-insemination is rare but has been shown to occur, and can readily be artificially induced to produce fertile eggs.

It might be argued that transference of a spermatophore from the seminal vesicle to the trunk of the same individual might occur during copulatory activities, whether accidentally or not. The chances of the seminal vesicle coming into contact with another part of its own body would seem much lower than of touching the other animal. On one occasion only, a single mature individual in an aquarium was seen to be violently twisting its body as in the copulatory sequence. There was, however, no spermatophore relocation.

The obvious method by which two planktonic chaetognaths could become attached for copulation is by use of their grasping spines, since their other append-

ages, the fins, contain no musculature (Ghirardelli, 1968). We do not know whether an act of cannibalism is behaviorally different from copulation. When an individual seizes an animal smaller than itself, the wider spread of its spines enables it to encircle and render ineffective the spines of the smaller animal. When animals are of similar size, as two mature animals would be, it would be much more difficult for one to gain the advantage. In the ensuing struggle, spermatophores may be transferred and the animals may separate because neither can get a decisive grip on the other. In support of this possibility that copulation and attempted cannibalism are different results of the same process, we have occasionally seen mature animals in aquaria which have just ingested another chaetognath which appeared to be of similar size, where the attacker bore a spermatophore on its body. This strongly suggested that both copulation and cannibalism had occurred.

Reeve (1966) noted that he had never observed cannibalism in *Spadella cephaloptera* and this observation still holds true in observations of the breeding population which we maintain in this laboratory. He suggested that the benthic *Spadella* might have developed a mechanism to prevent self-predation based on recognition of different kinds of swimming vibrations, since it congregated on surfaces rather than being dispersed in a 3-dimensional space where there was less chance of encountering a member of the same species rather than a food organism such as a copepod. Since copulation in *Spadella* is achieved with the aid of adhesion of one of the pair to the substrate, the mechanisms of cannibalism are not necessary for copulation.

The distinction between *Spadella* and the planktonic chaetognaths is not absolute. *Sagitta hispida*, *S. helenae* and possibly other neritic species possess the ability to attach to surfaces such as the aquarium wall. Unlike *Spadella*, however, where the newly hatched larvae attach on hatching and 95% of the population is usually attached at any one time, *Sagitta hispida* does not attach significantly until approaching maturity and even then most of the population is usually to be found swimming in the water column, in aquaria. Egg masses on aquarium walls indicate that *S. hispida* utilizes surfaces for egg-laying, but we have not as yet seen indications that this species can transfer spermatophores while attached to a surface.

Ghirardelli (1968) was also able to induce self-insemination in *Spadella cephaloptera* although he had no evidence that it could occur unaided, and his observations of sperm migration in this region suggested that it was unlikely. Our observations suggest also that it is unlikely, but that it can occur. In *Sagitta setosa*, on the other hand, Dallot (1968) maintained isolated individuals in which 50% of the spawnings gave fertile eggs. He did not observe any phenomena indicative of cross-fertilization, although he did not discount its existence in that species.

Since *Sagitta hispida* is the only planktonic species currently being routinely raised over its whole life cycle in the laboratory, it is clearly too early to make generalizations covering the entire range of planktonic Chaetognatha with respect to relative frequency of cross- and self-fertilization. If, indeed, self-fertilization is a regular phenomenon which becomes more likely when opportunities for cross-fertilization are minimal, it might be expected to occur more frequently in species habitually in lower densities such as typically oceanic and bathypelagic ones.

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SUMMARY

1. The first description is provided of the act of copulation of a planktonic chaetognath which leads to successful insemination and fertilization. It consists of a behavioral sequence in which partners maintain contact at their head ends while engaging in violent movements which result in the transfer of spermatophores.

2. Acts of self-insemination in the same species are also described, as well as experiments in which self-insemination and successful fertilization are induced.

3. The consequences of attachment of spermatophores to various parts of the body are described and compared to observations on the benthic genus *Spadella*.

4. Experiments showed 2% of batches of eggs laid by individuals isolated prior to maturity produced hatchings, suggesting that self-fertilization, although uncommon, does occur naturally.

5. Experiments indicated that one insemination was effective for only a single batch of eggs.

6. The similarities between copulation in *Spadella* and *Sagitta* are discussed and the possibility that self-fertilization presents a short-term survival mechanism for populations at low density is suggested.

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